AMENDMENTS TO THE SPECIFICATION

Please amend the title as follows:

-- Methods and Compositions for Activating ~~or Inhibiting~~ VEGF-D and VEGF-C--

Please replace paragraph [0027] with the following rewritten paragraph.

-- [0027] Figure 1 shows the scintillation proximity assay. ~~(a)~~ Figure 1A shows the [[S]] sequence of the peptide encompassing the site at which the VHD of VEGF-D is cleaved from the C-terminal propeptide (C-pro). In VEGF-D from 293 EBNA cells cleavage occurs between arginine 205 and serine 206 (arrowhead) (Stacker, S. A., K Stenvers, C. Caesar, A. Vitali, T. Domagala, E. Nice, S. Roufail, R. J. Simpson, R. Moritz, T. Karpanen, K. Alitalo, and M. G. Achen. 1999. Biosynthesis of vascular endothelial growth factor-D involves proteolytic processing which generates non-covalent homodimers. J. Biol. Chem. 274:32127-32136). Numbers above the amino acid sequence denote positions in human VEGF-D (Achen, M. G, M Jeltsch, E. Kukk, T. Mkinen, A. Vali, A. F. Wilcs, K Alitalo, and S. A. Stacker. 1998. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk-1) and VEGF receptor 3 (Flt-4). Proc. Natl. Acad. Sci. USA95:548-553). The C-terminal cysteine residue, not found in VEGF-D, facilitated radiolabeling. ~~(b)~~ Figure 1B shows the [[P]] principle of SPA. Black bars represent biotinylated (B), tritiated VEGF-D peptide that is treated with proteases, then bound to streptavidin conjugated scintillant beads prior to .beta.-counting. SB denotes scintillant beads and open brackets denote streptavidin moieties conjugated to the beads. ~~(c)~~ Figure 1C shows the [[S]] SPA results following treatment of VEGF-D peptide with proteases. Values are the average of three replicates ± one standard deviation and are representative of duplicate experiments. P values comparing plasmin- or thrombin-treated samples with negative control were calculated using Students' t test. Negative control is undigested peptide. ~~(d)~~ Figure 1D shows the [[M]] mass spectrometric analysis of VEGF-D peptide before ~~(upper panel)~~ and Figure 1E shows the mass spectrometric analysis of VEGF-D peptide after plasmin digestion ~~(lower panel)~~. Identity of the major peak in Figures 1D and 1E ~~each panel~~ is shown. --

Please replace paragraph [0028] with the following rewritten paragraph.

-- [0028] Figure 2 shows proteolytic processing of VEGF-D by plasmin--Western blotting. ~~(a)~~ Figure 2A shows the [[A]] analysis of human VEGF-D-FULL-N-FLAG (100 ng/lane) with anti-VHD antibody after digestion with 10, 1, 0.1 or 0 U/ml of plasmin. ~~(b)~~ Figure 2B shows α2-antiplasmin inhibition of plasmin. Plasmin (1 U/ml; 130 nM) was incubated with a range of α2-

antiplasmin concentrations prior to addition of VEGF-D-FULL-N-FLAG and incubation at 37°C for 1 hour. α2-antiplasmin:plasmin molar ratios are shown above the blot. ~~(c)~~ Figure 2C shows the [[A]] analysis of mouse VEGF-D isoforms (100 ng/lane). Mouse VEGF-D^{358} (358) and VEGF-D^{326} (326) were treated with 1 U/ml plasmin (+) or left undigested (-). Sizes of molecular weight markers in kDa are shown to the left of each panel.--

Please replace paragraph [0029] with the following rewritten paragraph.

--[0029] Figure 3 shows that mature growth factors generated by plasmin bind and cross-link receptors. ~~(a)~~ Figure 3A shows [[B]] binding to soluble receptors. Receptor-Ig fusion proteins containing the extracellular domains of human VEGFR-2 or VEGFR-3 were conjugated to protein-A sepharose and incubated with mature recombinant human VEGF-D as positive control (Mature), PBS as negative control, undigested and plasmin-digested full-length VEGF-D (Undigested and Digested, respectively). Upper, VEGFR-2 binding; Lower, VEGFR-3 binding. Material bound to the receptor-Ig proteins was analyzed by Western blotting using an anti-VHD antibody. Plasmin-generated mature VEGF-D (.about.21 kDa) is apparent. Sizes of molecular weight standards (kDa) are shown to the left. ~~(b) and (c)~~ Figures 3B and 3C show the [[A]] analysis of receptor binding and cross-linking in BaF3 bioassays. Ba/F3 cells expressing chimeric receptors containing the extracellular domains of VEGFR-2 or VEGFR-3 and the cytoplasmic domain of EpoR were treated with plasmin-digested or undigested full-length VEGF-D (Figure 3B) ~~(b)~~ or VEGF-C (Figure 3C) ~~(c)~~. ~~Upper panels:~~ Figures 3B and 3C show the VEGFR-2/EpoR bioassays. ~~Lower panels:~~ Figures 3D and 3E show the VEGFR-3/EpoR bioassays. Controls were medium lacking growth factor (*Medium*) or plasmin digests lacking growth factor (*Plasmin*). Values are the average of duplicates ± one standard deviation and are representative of three experiments. P values comparing results of plasmin-digested with undigested material were calculated using Students' *t* test.--

Please replace paragraph [0031] with the following rewritten paragraph.

[0031] Figure 5 shows both a VEGFR-2 bioassay ~~(left)~~ (Figure 5A) and a VEGFR-3 bioassay ~~(right)~~ (Figure 5B) with VEGF-C. Results with undigested full-length VEGF-C and material digested with plasmin are shown. VEGF-C was omitted to show the negative control. Values are the average of three replicates ± 1 standard deviation. P values for comparison of digested with undigested samples were calculated using Students *t* test.

Please replace paragraph [0057] with the following rewritten paragraph.

-- [0057] To identify proteases that activate VEGF-D, a scintillation proximity assay (SPA) was developed for monitoring cleavage of the C-terminal propeptide from the VHD. The assay was based on the C-terminal cleavage because this occurs at a single site in VEGF-D whereas cleavage of the N-terminal propeptide is more complex, occurring at two distinct sites (Stacker, et al., 1999, Biosynthesis of vascular endothelial growth factor-D involves proteolytic processing which generates non-covalent homodimers, J. Biol. Chem274:32127-32136). For the assay, a 17-mer peptide (containing residues 198 to 213 of human VEGF-D) spanning the C-terminal cleavage site of VEGF-D, was biotinylated at its N-terminus and radiolabeled at its C-terminus (Figure 1A [[a]]). The principle of the SPA is outlined in Figure 1B [[b]]. After the peptide is treated with proteases, it is bound to streptavidin-conjugated beads impregnated with scintillant. When the peptide is intact, the proximity of the radiolabel at the C-terminus of the peptide to the scintillant in the beads is sufficient to generate detectable photons. In contrast, there is a dramatic reduction in counts detected when cleavage of the peptide has occurred because the radiolabel is no longer sufficiently close to the scintillant for photons to be generated. --

Please replace paragraph [0058] with the following rewritten paragraph.

--[0058] A range of proteases were tested in this assay, including plasmin, thrombin and matrix metalloproteinase-2 (MMP-2) and MMP-9. These proteases were chosen because of their involvement in angiogenesis or tumor formation. MMP-2 and MMP-9 had no effect on the counts detected in the SPA, however, plasmin caused a greater than 90% reduction of signal, indicating substantial cleavage of the peptide (Figure 1C [[c]]).-- Thrombin caused a small reduction of signal. To identify the site(s) at which plasmin hydrolysed the peptide, samples were analysed by mass pectrometry. Undigested peptide consisted of a single peak of molecular mass 2282.15, as expected (Figure 1D ~~d, upper panel~~). Following plasmin treatment, a predominant peak of molecular mass 1267.68 was observed, corresponding to Biotin-HYSIIRR (Figure 1E ~~d, lower panel~~). This molecular species is an expected product of cleavage of the peptide at the same site as observed in VEGF-D expressed in 293EBNA cells, i.e. between R205 and S206 (Figure 1A [[a]]) (Stacker et al., 1999, J. Biol. Chem. 274:32127-32136). An alternative cleavage event generating Biotin-HPYSIIR (molecular mass 1111.59) was also detected.--

Please replace paragraph [0059] with the following rewritten paragraph.

--[0059] To establish if VEGF-D is a substrate for plasmin, this protease was incubated with full-length human VEGF-D (VEGF-D-FULL-N-FLAG) purified from the medium of transfected 293EBNA cells. A degree of proteolytic processing occurs in the medium of these cells resulting in VEGF-D preparations containing full-length material (~50 kDa) and a partially processed form (~31 kDa) consisting of the N-terminal propeptide and VHD (Figure 2A [[a]]) (Stacker, S. A., K. Stenvers, C. Caesar, A. Vitali, T. Domagala, E. Nice, S. Roufail, R. J. Simpson, R. Moritz, T. Karpanen, K. Alitalo, and M. G. Achen. 1999. Biosynthesis of vascular endothelial growth factor-D involves proteolytic processing which generates non-covalent homodimers. J. Biol. Chem. 274:32127-32136). After plasmin digestion, a single ~ 21 kDa band was detected by Western blotting (Figure 2A [[a]]). This species corresponds to the previously observed mature, fully-processed form of VEGF-D, suggesting that plasmin can cleave both the N-and C-terminal propeptides from the VHD. N-terminal amino acid sequencing of this ~21 kDa species revealed two sequences, FAATFYDIE and VIDEE, indicating that cleavage of the N-terminal propeptide was occurring at two sites. FAATF (residues 89 to 93 of VEGF-D) represents the sequence identified as the N-terminus of the predominant form of fully processed, mature VEGF-D purified from the conditioned medium of 293EBNA cells. VIDEE (residues 101 to 105 of VEGF-D) represents an N-terminus that is located one residue towards the C-terminus compared with that of the other form of mature VEGF-D (KVIDEE) detected in the medium of 293EBNA cells (Stacker, S. A., K. Stenvers, C. Caesar, A. Vitali, T. Domagala, E. Nice, S. Roufail, R. J. Simpson, R. Moritz, T. Karpanen, K. Alitalo, and M. G Achen. 1999. Biosynthesis of vascular endothelial growth factor-D involves proteolytic processing which generates non-covalent homodimers. J. Biol. Chem. 274:32127-32136). Therefore, plasmin cleaves the N-terminal propeptide from the VHD at almost identical positions to those described previously. In contrast to plasmin, the serine proteases thrombin and tissue plasminogen activator were unable to cleave the propeptides of human VEGF-D from the VHD (data not shown). --

Please replace paragraph [0060] with the following rewritten paragraph.

--[0060] The plasmin used in this study was purified from human plasma. In order to eliminate the possibility that the processing of VEGF-D observed was due to a contaminating activity in the plasmin preparation, α2-antiplasmin, a specific inhibitor of plasmin that forms an inactive 1:1 complex with this protease (Collen at al., 1991, Blood 78:3114-3124)), was incubated with the plasmin sample before digestion of VEGF-D. Analysis of resulting digestion products demonstrated complete inhibition of digestion by α2-antiplasmin when included at a 5-fold molar excess to plasmin (Figure 2B [[b]]). Therefore, the observed proteolytic processing of VEGF-D by the plasmin preparation used here was due to plasmin.--

Please replace paragraph [0061] with the following rewritten paragraph.

--[0061] Full-length mouse VEGF-D exists as two isoforms, VEGF-D_{326} and VEGF-D_{358}, that differ in the C-terminus of the protein (Baldwin et al., 2001, J. Biol. Chem. 276:44307-44314). Plasmin digestion of the mouse VEGF-D isoforms was carried out to analyze the effect of the distinct C-termini on proteolytic processing. Plasmin treatment of both isoforms produced a ~1 kDa species containing the VHD, as for human VEGF-D, indicating that this enzyme can fully process both isoforms (Figure 2C [[c]]).

Please replace paragraph [0063] with the following rewritten paragraph.

--[0063] To compare the capacities of full-length and plasmin-generated mature VEGP-D to bind and cross-link receptors at the cell surface, bioassays were employed utilizing Ba/F3 pre-B cells expressing chimeric receptors consisting of the extracellular domains of human VEGFR-2 or VEGFR-3 and the transmembrane and cytoplasmic domains of the eryturopoietin receptor (EpoR) (Stacker, et al., 1999, A mutant form of vascular endothelial growth factor (VEGF) that lacks VEGF receptor-2 activation retains the ability to induce vascular permeability, J. Biol. Chem. 274:34884-34892; Achen, et al., 2000, Monoclonal antibodies to vascular endothelial growth factor-D block interactions with both VEGF receptor-2 and VEGF receptor-3, Eur. J. Biochem. 267:2505-2515). These cell lines are IL-3-dependent, however, signaling from the EpoR cytoplasmic domain, that occurs when the extracellular domains of the chimeric receptors are cross-linked by ligand, leads to cell survival and proliferation in the absence of IL-3. These bioassays allow comparison of receptor binding and cross-linking and were used to define the receptor interactions of a range of VEGFR-2 and VEGFR-3 ligands (Achen, et al., 1998, Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk-1) and VEGF receptor 3 (Flt-4), Proc. Natl. Acad. Sci. USA 95:548-553; Stacker, et al., 1999, A mutant form of vascular endothelial growth factor (VEGF) that lacks VEGF receptor-2 activation retains the ability to induce vascular permeability, J. Biol. Chem. 274:34884-34892; Achen, et al., 2000, Monoclonal antibodies to vascular endothelial growth factor-D block interactions with both VEGF receptor-2 and VEGF receptor-3, Eur. J. Biochem 267:2505-2515; Wise, et al., 1999, Vascular endothelial growth factor (VEGF)like protein from orf virus NZ2 binds to VEGFR2 and neuropilin-1, Proc. Natl. Acad. Sci. USA 96:3071-3076). Cells were incubated with undigested or plasmin digested VEGF-D in the absence of IL-3 and the proliferative response assessed by incorporation of [^{3}H]thymidine into DNA. Cells expressing either the VEGFR-2 or VEGFR-3 chimeric receptors, that were exposed to plasmin-digested VEGF-D, exhibited a much greater response than those treated with undigested protein (Figure 3B [[b]]). Therefore, plasmin treatment generates mature forms of VEGF-D that are much better able to bind and cross-link VEGFR-2 and VEGFR-3 at the cell surface than full-length

material. Comparable results were observed with VEGF-C, (Figure 3C [[c]]), indicating that plasmin activates both of the known lymphangiogenic growth factors. --

Please replace paragraph [0066] with the following rewritten paragraph.

--[0066] This indicates that plasmin proteolytically released the VHD from full-length material and both of the partially processed forms. This effect was dose-dependent as, in comparison to the result with $1x10^{-4}$ units, the VHD became less abundant and the other species more abundant with decreasing concentrations of plasmin (Figure [[1]] 4, plasmin lanes 10^{-5} to 10^{-7}). In contrast, the effect of thrombin was marginal at best, even at the highest concentration of enzyme (Figure [[1]] 4., thrombin lanes 10^{-4} to 10^{-7}). These results demonstrate that plasmin activates VEGF-D as it can liberate mature VEGF-D from unprocessed and partially processed forms. --